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THE VALUE OF COLLODION MEMBRANES AS FILTERS.*

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MOST investigators have considered that the collodion membrane was only semipermeable, that it retained not only formed particles, but also to a greater or less degree soluble substances; and that according as these did or did not pass through the collodion sac some idea could be gained of the nature and size of their molecules. With this end in view, much work was done in filtering solutions of sugars, peptones, and proteins, and also of ferments, toxins, agglutinins, and lysins; the results obtained have however varied with the different investigators. The following experiments may explain these variations and assist in determining the value of the collodion membrane as a filter.

The sacs used in this work were made and mounted on glass tubes according to the Novy technic¹ with the modification of Gorsline;² they were about 2.5 inches in length and 0.5 inch in diameter. They were made by turning the tubes used twice in a dilute collodion; were without flaws or air bubbles, and of such thinness as to be practically invisible when placed in water; and they collapsed on emptying. After being mounted on glass tubes in a rubber stopper, they were placed in test tubes on a foot, provided with a side arm. The joint between the rubber stopper and the tube was air-tight. The sacs thus mounted were filled with distilled water, autoclaved at 105° for 15 minutes, allowed to cool, then emptied, immersed in sterile water, and subjected to air pressure (three inches of mercury). If there was no evidence of leakage, the filtration was commenced. This was carried on under a two-inch vacuum. After the filtration, the sacs were again emptied, immersed in water, and retested by pressure of three inches of mercury. If the sacs were still perfect, the filtrates were used for

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¹ *Laboratory Work in Bacteriology*, Ann Arbor, Wahr, 1899, p. 499.

² *Contributions to Medical Research Dedicated to Dr. V. C. Vaughan*, Ann Arbor, Wahr, 1903, p. 390.

inoculation experiments. In this manner diphtheria toxin and cobra venom were filtered.

DIPHTHERIA TOXIN.

Filtration of diluted diphtheria toxin.—The diphtheria toxin used was of such strength that 0.01 c.c. injected subcutaneously into a 250 gm. guinea-pig killed in 39 hours. The toxin was diluted 1 to 100 in 0.8 per cent sodium chloride. Three and one-half c.c. of this dilute toxin filtered through a collodion sac in 50 minutes. Whereas 1 c.c. of this dilute toxin unfiltered killed a guinea-pig in 39 hours when inoculated subcutaneously, the same dose of the filtrate caused only a slight induration at the point of injection with complete recovery at the end of a few days. Repetition of this experiment gave the same results. In this instance the collodion filter had retained the greater part of the toxin, thus showing agreement with the results of Rodet and Guecheff,¹ who found that diphtheria toxin did not pass through a collodion membrane.

Filtration of undiluted diphtheria toxin.—The same toxin undiluted was filtered through a collodion sac. The filtration was much slower, as it took four hours to filter through 2.5 c.c. On diluting the filtrate 1 in 100 with 0.8 per cent sodium chloride, 1 c.c. was injected subcutaneously into a guinea-pig and caused death in 38 hours. Apparently all of the toxin had passed through

TABLE 1.
FILTRATION OF DIPHTHERIA TOXIN THROUGH A COLLODION MEMBRANE.

Diphtheria Toxin	Subcut. Injec. in 250 gm. Guinea-Pig	Symptoms	Results
1. Dilute (1:100).....	1 c.c.	Very slight induration	Recovery at end of 2-3 days
2. Dilute (1:100).....	"	Induration	" " " 5 "
1. Undiluted.....	1 " diluted 1:100	Death within 38 hours	
2. Undiluted.....	" " "	" " 4.5 days	
Unfiltered.....	" " "	" " 39 hours	

the collodion sac. On repeating this experiment, some of the toxin was evidently retained, as the same dose caused death, but after 4.5 days. Here, it will be seen, the results are at complete variance with those obtained with the diluted toxin. Evidently the concentration is an important factor in the question of the semipermeability of the collodion membrane.

¹ *Compt. rend. soc. de biol.*, 1900, 52, p. 955.

COBRA VENOM.

Filtration of dilute cobra venom.—A solution of cobra venom was made in 0.8 per cent sodium chloride, of such strength that each cubic centimeter contained 0.2 mg. of the venom. Four and one-half c.c. of this solution were filtered through a collodion sac in one hour and a half. One and one-half c.c. of the unfiltered venom (0.0003), injected intraperitoneally into a guinea-pig, gave rise to immediate symptoms of excitement, followed in 33 minutes by convulsions, and terminating in death in one hour and twelve minutes. But the same amount of the filtrate failed to cause any symptoms when injected into another guinea-pig; evidently the collodion membrane had not permitted the venom to pass through.

TABLE 2.
RETENTION OF DILUTE COBRA VENOM BY COLLODION MEMBRANE.

	Intraperit. Injec. in Guinea-Pig	Symptoms	Results
Filtrate.....	1.5 c.c.	No symptoms	
Unfiltered.....	" "	Symptoms; excitement at once; slight convulsions at end of 17 minutes	Death in 1 hour and 12 minutes

Dilution—0.0003 cobra venom in 1 c.c. 0.8 per cent NaCl solution.

Successive filtrations of dilute cobra venom.—Another solution of the cobra venom was made, in which each c.c. contained 0.8 mg. of the venom. Successive quantities of this solution were filtered through the same collodion sac. Four c.c. of the solution were put in a collodion sac. After 3 c.c. had filtered through, the filtrate was removed and labeled Filtrate 1; the fluid remaining in the sac was carefully pipetted off, and all moisture was absorbed from the outside of the sac by filter paper. Then a second 4 c.c. of the original solution were placed in the same sac, 3 c.c. again filtered through, marked Filtrate 2, and again the sac was emptied and partially dried as before. This was done because the fluid remaining in the sac was probably somewhat concentrated, as the water passes through most rapidly; and in this experiment it was desirable to keep the concentration of the fluid unchanged, previous work on diphtheria toxin having shown variations in concentration to be an important factor in the collodion filtration. In this manner four successive quantities of the cobra venom were filtered

through the same sac. The filtrations became successively slower. At the end of the filtrations, the sac as usual was emptied, immersed in distilled water, subjected to air pressure (three inches of mercury), and found to be free from leaks. The unfiltered venom and the first, third, and fourth filtrates were then injected intraperitoneally into guinea-pigs. The unfiltered venom caused symptoms of intense excitement at once; convulsions commenced within 5 minutes and the animal died within 31 minutes. The first filtrate gave rise to no symptoms within the first two hours, but the animal was found dead next morning. The second filtrate was not tested. The third filtrate gave symptoms of excitement at once, followed by convulsions and death in 58 minutes. The fourth filtrate gave immediate symptoms of excitement, convulsions within the first 10 minutes, followed by death in 43 minutes after the injection. This experiment shows the gradual passage of the venom through the collodion membrane, the filtrates varying from those in which the greater part of the toxicity was lost, to those which differed but slightly in strength from the unfiltered venom.

TABLE 3.
GRADUAL PASSAGE OF DILUTE COBRA VENOM BY SUCCESSIVE FILTRATION THROUGH THE SAME
COLLODION MEMBRANE.

	Intraperitoneal Injection in Guinea- Pig	Symptoms	Results
1st filtrate (3 c.c.).....	1 c.c.	No symptoms of excitement or illness at once or in two hours	Found dead in 18 hrs.
2d filtrate (3 c.c.).....	Not tested		
3d filtrate (3 c.c.).....	1 c.c.	Symptoms of excitement at once	Death in 58 min.
4th filtrate (3 c.c.).....	"	Symptoms of excitement at once; convulsions at end of 10 minutes	Death in 43 min.
Unfiltered.....	"	Symptoms of excitement at once; convulsions at end of five minutes	

Dilution—0.0008 cobra venom in 0.8 NaCl solution.

SUMMARY AND CONCLUSIONS.

Dilute diphtheria toxin was retained by the collodion membrane, while the undiluted toxin passed through freely.

When dilute cobra venom was filtered, all toxicity was lost. On filtering successive quantities through the same collodion membrane, the filtrate gradually became toxic, until the fourth filtrate was practically of the same strength as the control. This result is

in accord with the work of Marbe¹ on the successive filtration of agglutinins through collodion sacs, and also with the gradual passage of complement through a Berkefeld filter as shown by me² and later found by Muir and Browning³ working on the same subject.

Evidently filtration through collodion sacs, as through Berkefeld filters, is a phenomenon of adsorption, the substances in solution passing through when adsorption has reached a certain degree. Formed particles, however, if able to pass through at all, would pass through more rapidly in the beginning of filtration, and later, as the pores become clogged, they would be retained, while the opposite would occur with soluble substances, which appear in the filtrate only after adsorption has become more or less complete.

Thus, by changing the concentration, the quantity to be filtered, or the thickness of the sac, results may be obtained varying from total retention to complete passage of the active substances through the collodion membrane.

¹ *Compt. rend. soc. de biol.*, 1909, 67, p. 809.

² *Jour. Med. Res.*, 1904, 13, p. 409.

³ *Jour. of Path. and Bact.*, 1909, 13, p. 232.